

AGP-Ca²⁺ binding is essential for pollen development and pollen tube growth



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Arabinogalactan-proteins (AGPs) are highly glycosylated cellwall proteins essential for the plant reproductive process. AGPs are extensively decorated with 90% of arabinogalactan polysaccharides composed of arabinose and galactose, and minor sugars such glucuronic acid (GlcA), fucose, and rhamnose. AGPs have been proposed to bind and store calcium through GlcA residues in a pH-dependent manner, serving as Ca²⁺ reservoirs in the apoplast. As the sugar moiety of AGPs seems crucial for their function, we explored the role of glucuronosyltransferases (GLCATs), responsible for GlcA presence on AGPs, during Arabidopsis reproduction. Previously, five GLCATs were shown to transfer GlcA specifically onto AGPs and *glcat14a glcat14b glcat14d* mutants showed a reduction in methyl GlcA substitution in AGP-enriched preparations that held less Ca²⁺ than wild-type plants in vitro.

Here, the physiological role of glucuronidated AGPs was investigated by phenotypic characterisation of mutants during Arabidopsis reproduction. Mutant plants presented reduced fertility, with shorter siliques and fewer seeds, owing to the presence of defective pollen grains that failed to germinate. Almost 40% of pollen grains collapsed between the uninucleate and bicellular pollen stages. The defective pollen grains were devoid of content and lacked an intine layer, as confirmed by the absence of cellulose with calcofluor staining. Immunofluorescence analysis of AGP epitopes confirmed the

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reduced levels of AGs and GlcA in mutants.

Our results highlight the functional importance of AGP glycosylation, emphasizing the crucial role of GlcA in pollen development and pollen tube growth. These findings support the hypothesis that Ca^{2+} binding by AGPs is required for successful reproduction.

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Short Bio

Sílvia Coimbra, Principal Investigator of the SPRED group - Sexual Plant Reproduction and Development - <https://www.fc.up.pt/agplab/>, which currently integrates the Associate Laboratory for Green Chemistry, LAQV Requite, <https://laqv.requimte.pt/>. Her research area focuses on the Biology of Reproduction in Plants. Her research work has attracted a high level of funding, coming not only from national sources (FCT) but also from international sources (Marie Skłodowska-Curie Actions, COST Actions, bilateral cooperation projects). The benefits of these funded projects have been important for her research group for the host institution that is FCUP, but they also extend to the students of this institution, more precisely from the Biology Department. All this work developed and established over more than a decade, sustains stable steps towards the future, with a strategy supported by a young, motivated and dynamic team.